Role of Lysosomes in the Pathogenesis of Splanchnic Ischemia Shock in Cats

By Thomas M. Glenn, Ph.D. and Allan M. Lefer, Ph.D.

ABSTRACT

Splanchic arterial occlusion (SAO) for 2 hours followed by release of the occlusion in cats produced a lethal shock state characterized by cardiovascular collapse. Release of the occlusion resulted in a 45% fall in mean arterial blood pressure within 15 minutes; postrelease survival of these animals was 46 minutes. The plasma of cats with SAO shock exhibited a 3- to 4-fold increase in activities of the lysosomal enzymes β-glucuronidase and cathepsin, accompanied by accumulation of a myocardial depressant factor. Plasma from cats treated with methylprednisolone (20 mg/kg) prior to occlusion did not have significant levels of myocardial depressant factor nor significant increases in plasma lysosomal enzyme activity. Furthermore, the fall in mean arterial blood pressure in steroid-treated animals was significantly smaller when the occlusion was released and postrelease survival time was significantly longer. Pancreatic lysosomes from cats with SAO exhibited a marked increase in fragility as indicated by a reduction in total lysosomal enzyme activity and an increase in the percent of free enzyme activity, compared to lysosomes from cats with sham SAO or steroid-treated cats. These data indicate that the biochemical and hemodynamic alterations present in SAO-induced shock may be related to the disruption of pancreatic lysosomes and that glucocorticoids can markedly alter the course of this shock, possibly by decreasing the sensitivity of pancreatic lysosomes to splanchnic ischemia.

ADDITIONAL KEY WORDS

pancreas methylprednisolone mean arterial blood pressure myocardial depressant factor cathepsin β-glucuronidase electron microscopy
number of shock states, including SAO shock
(7).

MDF has been shown to be a peptide having a molecular weight of 800 to 1000 (11). Previous studies have shown that either the activators or the precursors of MDF arise from the ischemic splanchnic region, probably from the pancreas (9), and are transported via the lymph to the systemic circulation (12). Although the precise cellular events which initiate the formation of MDF are not well defined, hypoperfusion of the splanchnic bed appears to be the common denominator of all of the shock states in which MDF has been found (7). It has also been suggested that activation of lysosomal hydrolases in the ischemic splanchnic region and subsequent release into the systemic circulation may play a role in the development of irreversibility in a number of shock states, including SAO shock (13). Furthermore, plasma accumulation of MDF is associated with significant increases in plasma lysosomal enzyme activities in both endotoxin (14) and hemorrhagic (15) shock.

Pharmacologic concentrations of glucocorticoids are known to protect in many forms of shock if given early in the shock state (7, 15). However, the mechanism of the protective action of steroids in shock has remained obscure.

The present investigation was undertaken to: (1) characterize the properties of the lysosomes of the pancreas during splanchnic ischemia shock; (2) to determine if any relationships exist between the activation of MDF and alterations in the properties of pancreatic lysosomes in splanchnic ischemia shock; and (3) to determine the effects of methylprednisolone, a glucocorticoid, on heart rate, arterial and venous blood pressures, MDF production, status of pancreatic lysosomes, and survival in splanchnic ischemia shock.

Methods

ANIMALS

Healthy, parasite-free adult cats of either sex weighing 2.4 to 3.7 kg were employed in this study. The animals were anesthetized by sodium pentobarbital (30 mg/kg iv). The right carotid artery, left femoral artery, and right femoral vein were cannulated, and mean arterial blood pressure (MABP), central venous pressure, and heart rate recorded continuously on a Beckman Type RB dynograph. All animals received heparin sodium (1,500 U/kg iv).

SPECIAL SURGICAL PROCEDURES

Splanchnic Arterial Occlusion

This procedure consisted of complete ligation (clamping) of the celiac, superior mesenteric and inferior mesenteric arteries. The clamps were removed 2 hours later, and MABP then declined rapidly. When the MABP had declined to 60 mm Hg, 25 to 35 ml of blood was drawn through the carotid arterial cannula, and the experiment was terminated. Blood samples to determine plasma lysosomal enzyme activities were taken at fixed intervals throughout the experiment. No blood or fluid replacement was given to replace blood from sampling. Postrelease survival time is defined as the time from removal of the arterial clamps to the time at which the MABP spontaneously declined to 60 mm Hg.

In some of the cats, splanchnic arterial occlusion was carried out as previously described, but the clamps were not removed at the end of the 2-hour occlusion period. At this time, 25 to 35 ml of blood was drawn for MDF determination, and the experiments were terminated. These cats are termed SAO (no release) cats.

A third group of cats was subjected to the same surgical procedures except that the arteries were isolated but not clamped. These animals are termed sham SAO cats.

Adrenalecromy

Four cats were bilaterally adrenalectomized under sterile conditions according to previously described techniques (16), given dexamethasone (1 mg/day) for the first 2 postoperative days, and maintained on 0.9% NaCl and Purina cat chow ad libitum for 13 days. At this time, the animals were anesthetized and a rapid pancreatectomy was performed.

Steroid-Treated Animals

Methylprednisolone (20 mg/kg iv) was given 30 minutes prior to splanchnic arterial occlusion or sham splanchnic arterial occlusion. An additional dose of the steroid (20 mg/kg) was injected intravenously at the end of the 2-hour occlusive period just prior to removing the arterial clamps. This second dose was given to maintain high blood levels of the steroid during the postrelease period.

Solu-Medrol generously supplied by the Upjohn Co., Kalamazoo, Michigan.
LYSOMES IN SHOCK

HOMOGENIZATION AND FRACTIONATION OF THE PANCREAS

Preparation of Lysosomal Fractions

At the termination of each experiment, the pancreas was rapidly excised, placed in cold sucrose solution (0.25M), and weighed at 4°C. A 1:10 (v/v) pancreatic homogenate was prepared using a low speed Waring blender for 15 to 20 seconds. The homogenates were then centrifuged at 4°C and 1000 X g for 10 minutes. The sediment, consisting primarily of nuclear material and cell debris, was discarded. The supernatant fluid was centrifuged in a Servall centrifuge at 15,600 X g for 20 minutes (318-000 g X min). An aliquot of the supernatant fluid was taken for the assay of /3-glucuronidase. The activity obtained from the S fraction is referred to as the "free" enzyme activity. The pellet (1), which consisted of lysosomes and other subcellular particles, was washed twice with cold 0.25M sucrose and then gently resuspended in 15 ml of cold sucrose (0.25M) in a Waring blender.

Thermal Activation of Bound Lysosomal Enzymes

The lysosomal suspension prepared from fraction L was incubated in a Dubnoff water bath shaker at 37°C for 150 minutes. Aliquots of the incubated suspension were taken at selected intervals (0, 30, 60, 90, 120 and 150 minutes) and assayed for /3-glucuronidase activity. This procedure resulted in the thermal activation (release of free enzyme activity) of the bound or intralysosomal enzyme activity. In addition, the time course for lysosomal disruption can be used as an index of the fragility of the population of lysosomes. The aliquots were then centrifuged at 15,900 X g for 20 minutes. The supernatant fluid which resulted from this centrifugation was taken for the assay of /3-glucuronidase. The activity obtained from the S fraction is referred to as the "free" enzyme activity. The pellet (1), which consisted of lysosomes and other subcellular particles, was washed twice with cold 0.25M sucrose and then gently resuspended in 15 ml of cold sucrose (0.25M) in a Waring blender.

Processing of Plasma for MDF Activity

Blood samples were centrifuged at 3000 X g for 20 minutes. The plasma was removed and placed inside dialysis tubing (Nojux tubing, viscose process, Union Carbide Corp., 30 mm flat width) at 4°C under a pressure of 300 mm Hg for 36 to 48 hours. Ten-ml aliquots of the ultrafiltrates were then lyophilized to dryness. The samples were then reconstituted to 20% of their original volume with distilled water and fractionated on a gel filtration column (Bio-gel P-7, 200-400 mesh) as previously described (11). Elution of the plasma ultrafiltrates from the column with Krebs-Henseleit solution minus glucose yielded five fractions read at 230 m\(\mu\) (release of free enzyme activity). These fractions contained negligible MDF activity.

Assays for MDF activity were carried out on isolated cat papillary muscles according to previously described techniques (18). Samples containing MDF activity induced a negative inotropic effect on the isolated cat papillary muscle, and the activities were expressed in MDF units. One MDF unit is equal to 18 decrease in the developed tension of the cat papillary muscle compared with the developed tension of the muscle in Krebs-Henseleit solution.

Chemical Determinations

Samples of plasma and lysosomal fractions (0.1 ml) were assayed for /3-glucuronidase activity according to the method of Talalay et al. (19) using phenolphthalein glucuronide as substrate. Specific activities are expressed as the number of micrograms of phenolphthalein liberated per milligram of protein per hour at 37°C.

The method of Anson (20) was employed to determine plasma cathepsin activity using bovine hemoglobin as substrate. Specific activities are expressed as the number of microequivalents of tyrosine released per milligram of protein per hour at 37°C.

Circulation Research, Vol. XXVII, November 1970
TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham SAO (8)</th>
<th>SAO (9)</th>
<th>SAO (no filter) (6)</th>
<th>Sham SAO + filteroid* (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mm Hg) (initial)</td>
<td>159 ± 6.3</td>
<td>156 ± 6.4</td>
<td>158 ± 6.6</td>
<td>157 ± 7.0</td>
</tr>
<tr>
<td>MABP (mm Hg) (at release or sham release)</td>
<td>142 ± 7.4</td>
<td>136 ± 6.8</td>
<td>140 ± 2.1</td>
<td>131 ± 9.7</td>
</tr>
<tr>
<td>MABP (mm Hg) (15 min after release)</td>
<td>131 ± 7.3</td>
<td>75 ± 4.2</td>
<td>†</td>
<td>128 ± 11.3</td>
</tr>
<tr>
<td>MABP (mm Hg) (end of exp)</td>
<td>109 ± 6.1</td>
<td>60g</td>
<td>†</td>
<td>107 ± 7.8</td>
</tr>
<tr>
<td>Postrelease survival time (min)</td>
<td>8</td>
<td>42.2 ± 6.0</td>
<td>†</td>
<td>170 ± 32.7</td>
</tr>
</tbody>
</table>

All values are means ± SE. Number in parentheses is the number of cats in each group.

*The steroid was methylprednisolone. †Experiments terminated just before release of clamps (120 minutes after occlusion). §Experiments terminated when MABP declined to 60 mm Hg. £Sham-operated cats were matched in time to their appropriate experimental groups.

Protein determinations were carried out employing a micro-biuret technique with the absorbance read at 300 nm and calibrated with micro-Kjeldahl determinations.

**Electron Microscopy**

Small sections of pancreatic tissue (2 to 3 mm²) were fixed in 4% cacodylate-buffered glutaraldehyde (pH 7.4). Forty-micron thick sections were then made from the larger sections on a freezing microtome and collected in cold cacodylate buffer. The sections were then incubated at 37°C in a Gomori medium (pH 6.5) for the presence of acid phosphatase (a lysosomal marker), rinsed in cold sodium acetate buffer (pH 6.5), and postfixed in cacodylate-buffered 1% osmium tetroxide for 1 hour according to the method of Miller and Palade (21). The sections were then dehydrated in ethyl alcohol and embedded in Epon. Ultra-thin sections (600Å) were prepared with uranyl acetate and lead citrate to enhance their contrast of the embedded sections. Lysosomal fractions (L) were treated in essentially the same manner as the pancreatic sections.

**Results**

A summary of the changes in systemic mean arterial pressure and postrelease survival times for all five groups of cats studied are shown in Table 1. Mean arterial blood pressure decreased slightly over the 2-hour occlusion period in all of the groups studied. However, release of the occlusion in SAO cats resulted in a significant (P < 0.001) fall of 45% in MABP; whereas SAO cats treated with methylprednisolone exhibited only a 17% decrease in MABP. Cats with sham SAO with and without steroid treatment had no significant changes in MABP during the same interval. The modification of the initial hemodynamic response to release in the shocked cat by methylprednisolone does not seem to be dependent on the ability of the steroid to exert any specific changes in hemodynamic function since the steroid produced no significant changes in MABP, central venous pressure, or heart rate.

In addition, previous steroid treatment of SAO cats resulted in a fourfold increase in postrelease survival (P < 0.001). Thus, the pharmacologic doses of methylprednisolone employed in SAO cats significantly modified the initial hemodynamic response to the release of the occlusion and also increased the postrelease survival time of these animals.

The plasma MDF activities for all five groups of cats are summarized in Figure 1. Previous studies have indicated that an inverse relationship exists between plasma MDF concentration and survival (7). This relation-
FIGURE 1

Bar graph of plasma myocardial depressant activity in column eluates expressed in MDF (myocardial depressant factor) units, obtained from all five groups of cats studied. Height of the bars indicates mean MDF activity, and SE is indicated. Number inside bars in number of samples measured. One MDF unit is equal to a 1% decrease in the developed tension of an isolated cat papillary muscle under standardized conditions (see Methods).

A relationship was also found in the present investigation. Whereas MDF activity in plasma from SAO cats was very high, that in plasma from steroid-treated cats subjected to the same degree of SAO was not significantly different from that found in sham SAO and steroid-treated sham SAO cats. In addition, plasma from cats subjected to SAO without release of the occlusion did not have significant MDF activity. Thus, MDF appears to be formed in the ischemic splanchnic region during the occlusive period and probably enters the circulation in large quantities only after release of the occlusion.

Two typical column elution patterns of plasma ultrafiltrates are illustrated in Figure 2. The upper panel shows that peak D eluted from an SAO cat contained essentially all the MDF activity (83 MDF units) present in the plasma. The peak D of an ultrafiltrate from a steroid-treated cat subjected to SAO was smaller in area and contained very low MDF activity (17 MDF units) compared with the SAO sample. No appreciable MDF activity was found in any of the other four peaks eluted from the ultrafiltrates in either sample.

Figures 3 and 4 summarize the alterations in plasma β-glucuronidase and cathepsin activities during SAO shock; both lysosomal enzymes showed similar plasma activity patterns. There was a significant increase in the activities of both enzymes within 15 minutes after arterial occlusion. This high level of activity was fairly well maintained over the 2-hour occlusive period. An additional rise in the plasma activities of these enzymes occurred following release of the occlusion. This second rise presumably indicates the washout of enzyme that has accumulated in the...
Graph of plasma β-glucuronidase activity in cats subjected to splanchnic arterial occlusion and sham splanchnic arterial occlusion. Occlusion of celiac, superior, and inferior mesenteric arteries at first arrow and release of the occlusion at the second arrow. Means and SE are shown.

Graph of plasma cathepsin activity in cats subjected to splanchnic arterial occlusion and sham splanchnic arterial occlusion. Occlusion of celiac, superior, and inferior mesenteric arteries is noted by the first arrow and release of the occlusion by the second arrow. Means and SE are shown.

Ischemic splanchnic bed during the period of occlusion. The rise in β-glucuronidase activity peaked within 30 minutes after release but the cathepsin activity continued to increase.
LYSOSOMES IN SHOCK

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial (0 min)</th>
<th>Preclamp (120 min)</th>
<th>Postclamp (135 min)</th>
<th>Terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham SAO</td>
<td>30.5 ± 3.4</td>
<td>55.0 ± 10.6</td>
<td>34.6 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>SAO (no release)</td>
<td>25.0 ± 9.2</td>
<td>66.7 ± 14.4</td>
<td>30.6 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>SAO</td>
<td>29.8 ± 5.3</td>
<td>75.5 ± 11.4</td>
<td>30.0 ± 8.7</td>
<td></td>
</tr>
<tr>
<td>SAO + methylprednisolone</td>
<td>27.0 ± 2.6</td>
<td>30.0 ± 3.1</td>
<td>29.0 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Sham SAO + methylprednisolone</td>
<td>29.5 ± 3.1</td>
<td>30.0 ± 4.6</td>
<td>30.0 ± 8.7</td>
<td></td>
</tr>
</tbody>
</table>

β-glucuronidase activity is expressed as micrograms of phenolphthalein × 10⁻² released per milligram protein per hour at 37°C. All values are means ± SE, N = the number of animals in each group.

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial (0 min)</th>
<th>Preclamp (120 min)</th>
<th>Postclamp (135 min)</th>
<th>Terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham SAO</td>
<td>7.1 ± 1.4</td>
<td>7.2 ± 1.2</td>
<td>6.4 ± 1.0</td>
<td>8.7 ± 2.5</td>
</tr>
<tr>
<td>SAO (no release)</td>
<td>5.5 ± 1.8</td>
<td>10.9 ± 0.9</td>
<td>11.8 ± 2.1</td>
<td>21.1 ± 3.7</td>
</tr>
<tr>
<td>SAO</td>
<td>5.9 ± 0.8</td>
<td>9.5 ± 1.2</td>
<td>11.8 ± 2.1</td>
<td>21.1 ± 3.7</td>
</tr>
<tr>
<td>SAO + methylprednisolone</td>
<td>4.8 ± 1.1</td>
<td>6.0 ± 1.9</td>
<td>4.8 ± 1.5</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Sham SAO + methylprednisolone</td>
<td>5.0 ± 0.8</td>
<td>6.0 ± 0.8</td>
<td>6.3 ± 1.1</td>
<td>3.3 ± 0.9</td>
</tr>
</tbody>
</table>

Cathepsin activity is expressed as milliequivalents of tyrosine × 10⁻⁴ released per milligram protein per hour at 37°C. All values are means ± SE. N = number of animals in each group.

Plasma activities for β-glucuronidase and cathepsin for all five groups of cats are summarized in Tables 2 and 3. Two hours after clamping, there was a significant rise in the activities of both lysosomal enzymes in plasma from SAO cats with and without release of the vascular clamps. The plasma activities of both enzymes were not significantly altered in the other three groups of animals at any of the times at which they were measured. Thus, methylprednisolone treatment prevented the increases in lysosomal enzyme activities in plasma during the entire course of SAO shock. The significant increases in lysosomal enzyme activity observed in SAO shock probably reflects the degree of lysosomal disruption in the ischemic splanchnic bed. Although splanchnic ischemia was produced by clamping of the splanchnic arteries, the steroid appeared to interrupt the normal response to ischemia, namely, the disruption of splanchnic lysosomes with the subsequent release of their enzyme content into the systemic circulation.

To relate possible changes in pancreatic lysosomes with the biochemical and hemodynamic changes in SAO shock, lysosomal fractions of pancreas were prepared and studied. Figure 5 represents electron micrographs which show the presence of lysosomes in the fractions isolated from the normal cat pancreas. A, an electron micrograph of a portion of a pancreatic acinar cell, depicts lysosomes containing the stained acid phosphatase particles, interspersed with zymogen granules, and distributed in a perinuclear fashion. An electron micrograph of a "lysosomal pellet" prepared from the supernatant fraction of a pancreatic homogenate is shown...
Electron micrographs of a portion of an acinar pancreatic cell (A), supernatant fraction of tissue homogenate (B and C) and same fraction incubated for 120 minutes at 37°C (D). Magnifications: A, B and D, 25,000×; C, 76,880×. All sections were fixed in glutaraldehyde and incubated for acid phosphatase. Acid-phosphatase-positive granules can be seen scattered in some of the ribosomes of the rough endoplasmic reticulum and concentrated in the lysosomes. The lysosomes of the pancreas are similar in appearance to lysosomes in other tissues. The acid-phosphatase-positive granules can be seen in the homogenate fraction within intact lysosomes. After the lysosomes were disrupted by thermal agitation (D), no acid phosphatase can be seen within "ghost" membranes.

in B. The pellet contains several lysosomes, zymogen granules, a smaller number of mitochondria, and other subcellular particles. C is an electron micrograph of the lysosomal pellet shown in B photographed at higher magnification. The five lysosomes in this micrograph are characterized by the intensity and distribution of the acid-phosphatase positive particles within the lysosomes and the vacuoles which are prominent in several of the lysosomes pictured. To disrupt the lysosomes and release their internal enzymes, the lysosomal pellet was subjected to a thermal activation test. D is an electron micrograph of the same lysosomal pellet after it was subjected to the disruptive effect of 130

Coronary Res., Vol. XXVII, November 1970
LYSOSOMES IN SHOCK

Graph showing typical disruption curves for lysosomal pellets. The lysosomal suspensions were incubated at 37°C for 150 minutes to release bound enzymes from intact lysosomes. Thermal activation of bound enzymes occurred in 60 minutes within the pellet from a sham SAO cat compared with 120 minutes in the pellet from a methylprednisolone-treated sham SAO cat. Only 65% of the bound lysosomal enzyme was released in the steroid treated cat at 60 minutes of incubation at a time when lysosomes of sham SAO cat were maximally released.

Thermal activation of lysosomes results in the release of intralysosomal enzymes, the rate of which serves as an index of their fragility. Figure 6 shows the rate of release of β-glucuronidase from a lysosomal pellet. Maximal release of β-glucuronidase activity occurred much more rapidly in the lysosomal pellet obtained from a sham SAO cat than that from a steroid-treated sham SAO animal. After 60 minutes of incubation, maximal enzyme release had taken place in the pellet from the untreated cat. At this time, however, only 65% of the bound β-glucuronidase activity had been liberated from the pellet obtained from the methylprednisolone-treated cat. Expressed differently, only 10 minutes of incubation were required to release 50% of the β-glucuronidase activity from the sham SAO lysosomal pellet, whereas 30 minutes were required to release 50% of the enzyme activity in the pellet from the steroid-treated sham SAO cat. Thus administration of methylprednisolone in vivo appears to markedly increase the stability of pancreatic lysosomes.

The time required for peak release of β-glucuronidase activity from the lysosomal pellets in the five groups of animals studied are summarized in Figure 7. There are no significant differences in the time to peak enzyme release from the lysosomal pellets obtained from the three groups of animals not given steroid (i.e., sham SAO, and SAO with and without release). However, methylprednisolone treatment in either sham SAO or SAO cats markedly increased the time required for
TABLE 4
Influence of Splanchnic Arterial Occlusion, Methylprednisolone, and Adrenalectomy on Pancreatic Lysosomal Enzyme Activity

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>$\beta$-glucuronidase activity</th>
<th>Free</th>
<th>Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham SAO</td>
<td>5</td>
<td>100.0 ± 7.0</td>
<td>13.0 ± 2.0</td>
<td>86.0 ± 3.8</td>
</tr>
<tr>
<td>SAO (no release)</td>
<td>4</td>
<td>38.7 ± 7.9</td>
<td>27.0 ± 3.2</td>
<td>71.0 ± 2.4</td>
</tr>
<tr>
<td>SAO</td>
<td>5</td>
<td>53.7 ± 5.7</td>
<td>27.4 ± 3.5</td>
<td>72.6 ± 3.7</td>
</tr>
<tr>
<td>SAO + methylprednisolone</td>
<td>5</td>
<td>60.9 ± 6.5</td>
<td>13.8 ± 3.6</td>
<td>86.1 ± 3.5</td>
</tr>
<tr>
<td>Sham SAO + methylprednisolone</td>
<td>5</td>
<td>52.2 ± 6.6</td>
<td>16.9 ± 2.2</td>
<td>83.1 ± 2.2</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>4</td>
<td>103.8 ± 12.8</td>
<td>23.3 ± 2.9</td>
<td>76.7 ± 2.5</td>
</tr>
</tbody>
</table>

$\beta$-glucuronidase activity is expressed as micrograms of phenolphthalein $\times 10^{-2}$ released per milligram protein per hour at 37°C. N = number of animals in each group. All values are means ± SE.

Total pancreatic $\beta$-glucuronidase content as well as extralysosomal (free) and intralysosomal (bound) fractions were calculated. The values obtained are shown in Table 4. A significant increase in free $\beta$-glucuronidase activity occurred in animals subjected to SAO with and without release. In addition, there was a marked decrease in the total pancreatic $\beta$-glucuronidase activity in these two groups of SAO cats compared to the total activity observed in sham SAO cats. The decreased total pancreatic enzyme activity present in the SAO cats can probably be accounted for by the marked increase in plasma lysosomal enzyme activity seen in these cats. Thus, a large number of these lysosomes had already released much of their enzymes during shock and could therefore not release as much as lysosomes from nontraumatized cats.

Methylprednisolone pretreatment of the SAO animals prevented the increase in free enzyme activity. In fact, free lysosomal enzyme activity in both groups of steroid-treated animals was not significantly different from that seen in sham SAO cats. The total pancreatic lysosomal enzyme activity of both groups of steroid-treated cats was significantly lower than that seen in cats not treated with steroids, regardless of whether they were subjected to shock. This suggests that methylprednisolone increased the stability of the pancreatic lysosomes, and maximal release of enzyme activity could not occur in these lysosomes.

Since methylprednisolone altered some of the basic characteristics of pancreatic lysosomes, pancreatic lysosomes obtained from chronically adrenalectomized cats were also studied. The data indicate that pancreatic lysosomes obtained from adrenalectomized cats are less stable than normal. This is indicated by the fact that the percent of free $\beta$-glucuronidase was 67.6% higher than in normal cats even though the total $\beta$-glucuronidase activity was unchanged. Quantitatively, the free pancreatic $\beta$-glucuronidase activity in adrenalectomized cats was comparable to that found in the pancreatic lysosomes of cats after splanchnic arterial occlusion. These findings indicate that large doses of exogenous glucocorticoid can markedly alter the response of pancreatic lysosomes to splanchnic ischemia shock, whereas chronic deprivation of glucocorticoids can result in an increased fragility of pancreatic lysosomes.

Moreover, incubation of isolated pancreatic
lysosomes from control cats with concentrations of methylprednisolone equivalent to those seen in steroid-treated cats ($5 \times 10^{-4} \text{mg}$) resulted in a degree of lysosomal stabilization comparable to that seen in the intact animal. Thermal activation of the in vitro steroid-treated lysosomes yielded $\beta$-glucuronidase activity ($31.0 \pm 3.5 \text{ U/mg}$, mean $\pm \text{SE}$) which was only a third of that seen in lysosomes treated with the steroid vehicle ($91.1 \pm 17.9 \text{ U/mg}$). The total enzyme activities obtained from vehicle- and steroid-treated lysosomes in vitro were equivalent to those found in the lysosomes of sham SAO and steroid-treated sham SAO animals. These data indicate that methylprednisolone can stabilize pancreatic lysosomes in vitro.

**Discussion**

The data obtained from this study suggest that activation of lysosomal hydrolases in the pancreas is related to the production of a cardiotoxic factor which contributes to the cardiovascular collapse observed following occlusion of the arterial vessels supplying the splanchnic bed.

Previous studies of the pathogenesis of SAO shock have implicated a number of factors that may contribute to the lethality of this shock state. Kobold and Thai (22) have described the appearance of a vasoconstrictor peptide in the plasma of animals following occlusion of the superior mesenteric artery and suggested that its formation may be the result of the proteolytic action of pancreatic enzymes. Other investigators have suggested that the release of bacterial endotoxins from the ischemic intestine is involved in the pathogenesis of SAO shock (2, 3, 5, 23). Still others have attributed the lethality of SAO shock to such diverse factors as hypovolemia (4), release of vasoactive compounds such as serotonin, substance P, adenosine nucleotides (24), and proteolytic enzymes (25). However, none of the above factors has been satisfactorily implicated in the fatal cardiovascular failure of SAO shock.

Recently, several investigators (1, 2, 26) have observed that a pronounced myocardial impairment (as evidenced by a significant decrease in cardiac output without alterations in cardiac filling pressures) develops soon after occlusion of the superior mesenteric artery and that the intensity of the cardiac impairment progresses with continued occlusion. Furthermore, release of the occlusion itself seems to hasten the cardiovascular collapse.

Thus Williams et al. (26) demonstrated a negative inotropic factor in the plasma of dogs subjected to superior mesenteric artery occlusion which they suggested as the primary cause of the observed decrease in cardiac output. They implicated the ischemic gut as the site of release or production of the factor. The factor isolated by Williams produces a negative inotropic response similar to that obtained with MDF, and may be MDF.

The production of MDF also occurs in a number of shock states, including those due to hemorrhage, endotoxin, and pancreatitis in which the common denominator is hypoperfusion of the splanchnic bed (7). Lefer and Martin (9) have suggested that MDF may arise from the ischemic pancreas.

Although splanchnic hypoperfusion appears to be the initial stimulus for MDF production, the actual cellular changes leading to the production of MDF, its activators, or its precursors have not been clearly defined. Blockade of the celiac ganglia by local anesthetics (3) has been shown to reduce the severity of the shock produced by superior mesenteric artery occlusion. Wangensieen et al. (14) have also demonstrated that celiac ganglionic blockade results in the maintenance of normal levels of splanchnic blood flow and prevents the increase in plasma lysosomal enzymes as well as the plasma accumulation of MDF in endotoxin shock.

Recent studies have shown that increased plasma lysosomal enzyme activity in shocked animals correlates well with the accumulation of MDF in the peripheral blood (12, 14, 15). Although many investigators (12-14, 23, 25) claim that lysosomes play an important role in the cellular changes leading to the irreversibility of shock states, no convincing demonstrate...
tion of a direct toxic effect of lysosomal enzymes has thus far been reported. Thus lysosomes of the splanchnic region are known to be more sensitive to ischemia than the lysosomes of other regions of the body (27); and splanchnic ischemia has been shown to disrupt splanchnic lysosomes (23). Tissue ischemia and the resulting hypoxia and acidosis may be responsible for the activation and release of lysosomal enzymes (13). This hypothesis was advanced by deDuve (28), who found that acid conditions accelerated the release of hepatic lysosomal enzymes. The activity of the released hydrolases was found to be optimal at pH 5 (29), a condition approximating that found in ischemic tissue (30).

The data presented in this study show that significant changes in lysosomal enzyme activity and lysosomal integrity take place in pancreatic lysosomes during the course of SAO shock. These changes appear to be temporally related to the subsequent biochemical and hemodynamic alterations occurring after splanchnic arterial occlusion. The rapid increase in plasma hydrolase activity indicates the high degree of sensitivity exhibited by the splanchnic lysosomes to ischemia. Witte et al. (30) showed that splanchnic lymph Po2 falls to less than 8 mm Hg within 10 to 15 minutes of occlusion of the splanchnic vessels. The fact that the total pancreatic lysosomal enzyme activity of the SAO cats was 54% of that of sham SAO cats probably reflects the increased plasma enzyme activity in the SAO cats. The increase in lysosomal protease activity in the plasma before the plasma appearance of MDF supports this contention. Although plasma lysosomal enzyme activities were significantly elevated for most of the occlusive period, no significant MDF activity was found in the plasma of SAO cats in which the occlusion was not released.

There are several possible explanations for these findings. The lysosomal enzymes, and presumably preformed MDF, are transported to the systemic circulation either via splanchinic collaterals or via the lymphatic system. Since it would be difficult to transport the large molecular weight enzymes across capillary membranes, they would more likely be transported by lymphatic vessels. We have previously demonstrated significant lymphatic transport of lysosomal enzymes in hemorrhagic shock (12). Furthermore, Nelson et al. (31) showed a transient spikelike increase in lymph flow immediately upon initiation of hemorrhage. Lymph flow then gradually declined (12, 31) upon a sustained splanchnic ischemia resulting from prolonged oligemia. In the present study, the rapid increase in plasma lysosomal enzyme activity may have been due to this initial increase in lymph flow. In this regard, intravenous injection of pancreatic lysosomal enzymes simulating activities observed in SAO shock was sustained over a 2-hour period in two normal cats. This corresponds to the period of complete occlusion in the SAO cats.

In these cats, a large increase in β-glucuronidase activity was observed within 1 minute of intravenous injection of an aliquot of a pancreatic lysosomal pellet. This increased level of enzyme activity was maintained for 2 hours and decreased only 38% from the peak value in that time. No significant changes in MABP, central venous pressure, or heart rate occurred in these cats. These data indicate that the β-glucuronidase activity can be maintained in the plasma over periods such as those occurring in the SAO experiments. These findings support the hypothesis that an initial surge of lysosomal enzyme activity into the plasma could be maintained for the period of splanchnic arterial occlusion.

The transport of lysosomal enzymes and MDF via the lymphatic system leaves the question of the lack of high plasma MDF activities unanswered. There are several possibilities for the low plasma MDF activities in SAO cats prior to release of the occluded vessels. One possibility is that there is a substantial latent period between the release of lysosomal enzymes and the formation of MDF. In this regard, a latent period of 2 to 3 hours between splanchnic ischemia and MDF production has been demonstrated in shock...
LYSOSOMES IN SHOCK

Another possibility for the failure to accumulate MDF in the plasma may be that the pH of normal systemic blood (i.e., 7.3 to 7.4) is too far removed from the optimal pH of these enzymes (i.e., pH of 5.0 to 5.5).

A third possible reason for the lack of MDF in systemic plasma may be that MDF, even if it is formed, may be rapidly inactivated or removed by the normally functioning kidneys and reticuloendothelial system. These considerations support the hypothesis that MDF is produced within the splanchnic region during splanchnic ischemia shock and that conditions are not optimal for its production or accumulation in peripheral plasma during SAO shock.

Further support for the hypothesis that MDF is produced within the splanchnic region can be derived from the work of Williams et al. (26). These workers found that a myocardial depressant factor appeared in the plasma of dogs during the occlusive period of splanchnic ischemia shock. In their model, only the superior mesenteric artery was occluded, whereas in the present study, the celiac and superior and inferior mesenteric arteries were occluded. The possibility exists that when only the superior mesenteric artery is occluded, sufficient collateral circulation developed to ensure the transport of MDF from the splanchnic bed into the systemic circulation. In the SAO shock model employed in this study, both of these potential collateral pathways were occluded.

The large decrease in MABP after release of the occlusion may have resulted from the washout of large quantities of MDF or some other agent into the systemic circulation. Another possibility is that the splanchnic vasculature, already diluted by vasoactive agents, becomes engorged with blood after release of the occlusion. These agents may arise from the ischemic pancreas, liver, or intestine.

Data are available to indicate that MDF can rapidly and severely impair myocardial contractility. Thus addition of a bolus of MDF directly into the coronary perfusion medium of an isolated perfused cat heart decreases cardiac contractility by 83% within 1 to 2 minutes (8).

Some workers have attributed the protective effect of glucocorticoids in shock to an alleged direct positive inotropic effect on the heart (33). Lefer (34) found that cortisol did not increase the contractile force of either the isolated cat papillary muscle or the isolated perfused cat heart. Furthermore, Glenn and Lefer (15) were unable to demonstrate a significant increase in contractile force of the intact cat heart employing doses of methylprednisolone (20 mg/kg) equivalent to those used in the present study. Lillehei et al. (35) postulated that, in shock, glucocorticoids function as alpha-receptor blocking agents (i.e., vasodilators) but did not provide evidence for this mechanism. Others have suggested that the glucocorticoids are beneficial in shock because they potentiate the vasoconstrictor effect of the catecholamines (36). However, Kadowitz and Yard (37) recently showed that doses of cortisol which significantly protect animals subjected to endotoxin shock did not enhance the cardiac response to catecholamines, produce vasodilation, or act as an alpha-receptor blocking agent. These investigators proposed that cortisol might protect by altering the response of lysosomal enzymes to endotoxin administration.

Weissman and Thomas (17) have demonstrated the abilities of glucocorticoids both in vivo and in vitro to stabilize lysosomes in endotoxin and splanchnic ischemia shock. Glenn and Lefer (15) have proposed that the protective effect of methylprednisolone in hemorrhagic shock is based on the ability of the steroid to stabilize splanchnic lysosomes, since methylprednisolone-treated cats subjected to hemorrhagic shock had normal plasma lysosomal enzyme and MDF activities and
survived longer than vehicle-treated animals with shock.

The total enzyme activity of the in vitro methylprednisolone-treated lysosomal fractions was similar to that observed in lysosomes obtained from intact cats treated with steroid, whereas the portion of control pancreas incubated with an equivalent volume of methylprednisolone vehicle yielded fragilities and total activities comparable to those found in lysosomes obtained from cats with sham SAO. These findings are in agreement with those of other investigators (13, 17), who demonstrated the ability of glucocorticoids to reduce the fragility and free enzyme activity of lysosomes isolated from animals subjected to shock.

Depletion of endogenous glucocorticoids by chronic adrenalectomy resulted in an increase in lysosome fragility and a higher percent of free activity than in lysosomes from normal cat pancreas. The increased fragility of these lysosomes may provide a partial answer to the increased sensitivity of adrenalectomized animals to various forms of shock (13). Similar increases in free lysosomal enzymes in the tissues of adrenalectomized animals have been previously reported (38).

Acknowledgment

The authors wish to acknowledge the competent technical assistance of Mr. Julian B. Martin, Mr. Thomas F. Inge, Jr., and Mrs. Nancy I. Kelly during the course of this project.

References


Role of Lysosomes in the Pathogenesis of Splanchnic Ischemia Shock in Cats

THOMAS M. GLENN and ALLAN M. LEFER

*Circ Res.* 1970;27:783-797
doi: 10.1161/01.RES.27.5.783

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1970 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/27/5/783

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/